Determining molar extinction coefficient by high-accuracy optical saturation measurements

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ABSTRACT

Linear spectrophotometric transmittance measurements are widely used for determining the value of molar extinction coefficient and other basic photophysical parameters of dissolved molecular species. However, such measurements usually require prior information about the molar concentration of the studied chromophores, which in many cases such as e.g. genetically encoded self-maturing species, is not readily available. Here we use wavelength-tunable femtosecond pulses to demonstrate that by performing high-accuracy measurement of small intensity-dependent changes induced in the sample transmittance due to absorption saturation we are able to estimate the extinction coefficient without prior knowledge of the concentration.

Keywords: Nonlinear transmittance, molar extinction, optical absorbance, femtosecond pulses

1. INTRODUCTION

Molar extinction coefficient (ε_M) and closely related one-photon absorption cross section (σ_{1PA}) characterize the ability of a molecular species to absorb light and as such are widely used in various spectrophotometric measurements. A common application consists in determining chromophore molar concentration (C_M) using Beer-Lambert law, provided that the optical path length (d) and the value of ε_M of the solution are known. Or vice versa – ε_M may be determined from measured value of optical density ($O.D.$) or linear transmittance (T_{lin}) and C_M. However, researchers may also encounter circumstances, where neither ε_M nor C_M values can be readily determined. Such situations occur e.g. with genetically encoded chromoproteins that mature inside living cells or when a compound of interest has only very limited solubility or its physical state is unsuitable for accurate mass determination. Therefore, a practical methodology that would allow determining ε_M (or σ_{1PA}) independent of any prior knowledge of the corresponding C_M would be valuable.

While measurements of the $O.D.$ (or T_{lin}) and d are generally straightforward, a number of approaches have been proposed to address the issue of determining the values of C_M and ε_M in a mutually independent manner. These experiments commonly involve additional spectroscopic measurements typically utilizing some nonlinear optical effects such as e.g. saturation of absorption [1-3] or four-wave mixing [4]. Recently, Cho *et al.* have described a method for simultaneous all-optical determination of molecular concentration and extinction coefficient based on femtosecond two-beam pump-probe spectroscopy [5,6]. Their approach is based on determining the number of optically excited molecules in the solution, from which the value of C_M may be deduced, provided that both the pump- and the probe pulse have well-characterized photon flux accounting for the corresponding spectral- and temporal profiles. Even though such two-beam method appears reliable, it is associated with a considerable level of technical sophistication which may not be accessible outside of dedicated laboratories performing precision nonlinear laser spectroscopy.

In this paper we are proposing an alternative approach, which uses only one femtosecond laser beam thus eliminating need for precision beam alignment. Our method also relies on readily available linear absorption reference standards, which greatly alleviates the pulse characterization requirements. We make use of optical saturation phenomenon i.e. the fact that when incident photon flux (I_{in}) is sufficiently high, then the ratio between the transmitted (I_{tr}) and incident photon flux increases with increasing I_{in}. We also note that while the value of non-saturated or linear transmittance, T_{lin}, is a function of the product of the two parameters, $C_M\,\varepsilon_M$, the rate at which nonlinear transmittance, T_{NL}, changes due to the saturation,

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has a different functional dependence on these two parameters [1,2]. Thus, performing, in the same sample solution, the measurements of T_{lin} and T_{NL}, allows, in principle, independent determination of C_M and ε_M.

Main practical difficulty with this otherwise conceptually straight-forward approach consists in the well-known fact that the functional dependence of T_{NL} on C_M and ε_M is, in a general case, rather involved and therefore poorly suited for accurate determination of the named parameters [7]. However, if the absorbance of the specimen is low, i.e. linear transmittance is close to unity, $1 - T_{lin} < 0.1$, and if the corresponding change caused by the saturation effect is also relatively small, $(T_{NL} - T_{lin})/(1 - T_{lin}) < 0.1$, then the named functional dependence becomes approximately linear, which greatly simplifies determination of C_M and ε_M from the experimental data. Of course, such "linearized" saturation regime implies that T_{NL} needs to be measured with sufficiently high accuracy, for which we take advantage of advanced nonlinear transmittance techniques that were recently developed for measuring two-photon absorption spectra [8], and which we modify here for our current purposes.

Below we describe an experiment, where we use wavelength-tunable femtosecond pulses to measure nonlinear transmittance spectrum of Rhodamine 800 dissolved in ethanol in the range of the $S_0 \rightarrow S_1$ electronic transition, $\lambda = 640 - 735$ nm, with accuracy better than 0.1%. By keeping the linear absorbance of the solution low, $1 - T_{lin} < 0.05$, and also by restricting the change of saturation to the linear regime, we show that the measured value of T_{NL} in the samples with different C_M values scales linearly with the concentration, meaning that this approach could be used as a practical way of determining C_M and ε_M relative to some reference sample where named parameters are known when studied under identical excitation conditions.

2. EXPERIMENTAL

Figure 1 shows the experimental set-up used in our experiment. A Light Conversion Inc. laser system comprising a Pharos regenerative amplifier (maximum pulse energy 1 mJ at 1031 nm) pumping an Orpheus-HE parametric amplifier, where the latter generated 150 - 200 fs duration pulses are tunable in the wavelength range $\lambda = 640 - 735$ nm with the maximum pulse energy of 0.1 - 0.2 mJ. The laser pulse repetition rate was lowered from maximum 6 kHz to 100 Hz to minimize thermal artifacts in the solution. To further reduce potential thermal effect, the OPA output beam diameter was expanded to ~15 mm using a telescope (L1 and L2), and was then directed through a variable neutral density filter wheel (O.D. = 0.04 - 2.0) mounted on a computer-controlled stepper motor. The attenuated beam was focused with a long focal length lens (L3) (f = 1000 mm). The sample solution (S) was contained in 10 mm path length spectroscopic quartz cuvette placed at some distance after the lens L3 focus. The distance between the beam focus and the sample was adjusted and maintained by mounting the focusing lens on a computer-controlled 200 mm travel linear stage. Two uncoated glass plates (GP1 and GP2), located before and after the sample, reflected a small fraction of the beam to the two 50 mm diameter integrating spheres (IS1 and IS2, Thorlabs IS200)

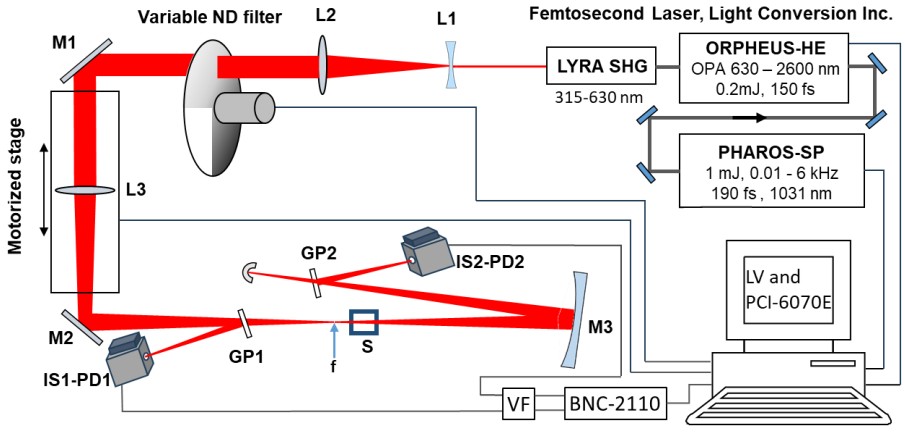

Figure 1. Measurement scheme. L1, L2 and L3 are beam shaping lenses. ND refers to neutral density. M1 and M2 are planar mirrors, M3 is Ø150 mm spherical mirror with 500 mm focal length. IS1-PD1 and IS2-PD2 indicate integrating spheres equipped with photodiodes. VF is voltage follower for impedance matching, BNC-2110 is terminal block connecting signal cables with NI PCI-6070E data acquisition board.

equipped with Thorlabs DET100A2 biased Si photodiodes (PD1 and PD2), which detected, respectively, the relative energy of the pulse incident on the sample and that transmitted through the sample. A spherical mirror (M3) with the focal length f = 500 mm was used to collect light that might be scattered due to some optical inhomogeneity occurring in the cuvette and/or in the sample. The voltage signals from the two photodetectors were passed through two-channel unity gain amplifier (voltage follower -VF) for impedance matching with digital data acquisition board (National Instruments DAQ PCI-6070E equipped with BNC-2110 terminal block). and then collected and averaged using a LabView-based program routine, which also controls the OPA wavelength, as well as the positions of ND filter wheel and the focusing lens L3. Linear absorption spectra were measured using a Shimadzu UV-3600 Plus spectrophotometer.

3. RESULTS AND DISCUSSION

Left panel of Figure 2 shows the linear i.e. unsaturated transmittance spectrum of Rhodamine 800 in ethanol (ε_M @ 682 nm = 113,3000 ± 6,000 M^{-1} cm^{-1} [9]) at two different dye concentrations (solid lines), where the higher-concentration sample (blue line) shows at the peak of the $S_0 \rightarrow S_1$ electronic transition the maximum transmittance change of 0.053, whereas the corresponding value of the lower-concentration sample (tan line) is about factor of 2 lower, 0.026. The nonlinear transmittance spectrum (dots) was measured in both samples by tuning the OPA wavelength in 5 nm steps in the range λ = 640 – 735 nm and by recording at each wavelength the nonlinear transmittance function, which was obtained as the ratio between the integrated pulse voltage signals of the PD1 (incident) and PD2 (transmitted) detectors. The right panel of Figure 2 shows an example of the measured nonlinear transmittance function obtained at 685 nm and normalized at the pulse energy, where transmittance starts to show clear nonlinear behavior. We call this "lowest acceptable input energy". When the incident pulse energy is increased from that lowest acceptable input energy value in small increments (by rotating the ND wheel), then the sample transmittance increases due to the saturation and the corresponding photodetector signal ratio, PD1 / PD2, decreases from its maximum value. This procedure is performed at each excitation wavelength individually meaning that both the initial raw transmittance as well as the lowest acceptable input energy may have different values at different wavelengths. If the pulse energy stays relatively constant in the scanned wavelength range, then in most experiments it is sufficient to vary initial raw transmittance while keeping lowest acceptable input energy unchanged for all wavelengths.

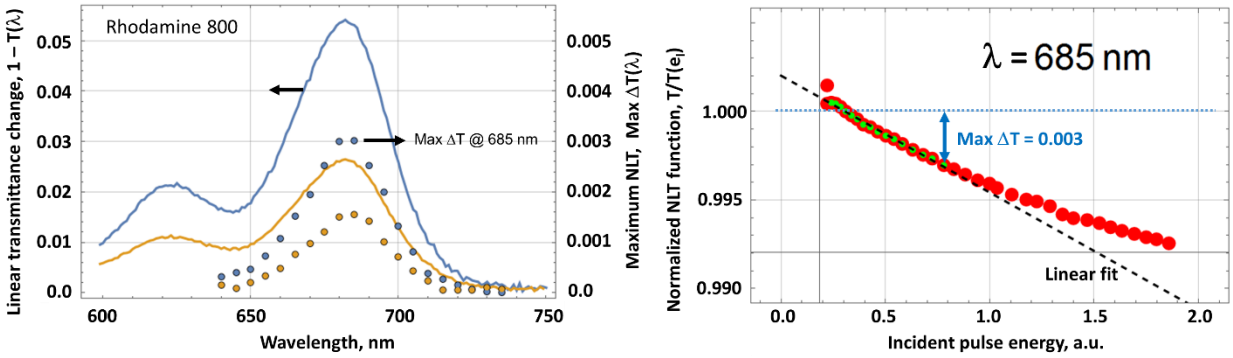

Figure 2. Left: measured linear transmittance spectra of Rhodamine 800 in ethanol at two different dye concentrations (solid lines) compared to their nonlinear transmittance spectra (dots) at λ = 640 – 735 nm. Blue colored graphs indicate higher concentration. Right: nonlinear (saturated) transmittance function of a higher-concentration sample at λ = 685 nm normalized at lowest acceptable input energy e_I. Red dots show the ratio of incident signal over transmitted signal with increased pulse power. Green dots show the range of quasi-linear change of that ratio fitted with linear function (black dashed line). Maximum difference (the slope parameter) of the nonlinear transmittance function at relative pulse energy of 0.8 is indicated by Max ΔT. The slope parameters at other excitation wavelengths form the dotted graphs on the left side of the figure.

As was discussed in the Introduction, when the transmittance changes are relatively small then the dependence of T_{NL} on the incident photon flux may be approximated with a linear function. The dashed black line in the figure shows such linear fit which was obtained for the data points in the quasi-linear range, Max $\Delta T_{NL} < 0.003$ (indicated by green dots), whereas the data points that deviate from the linear behavior (marked as red dots) were excluded from this fit. As the next step, the slope parameter of the fit function was evaluated at each excitation wavelength. The corresponding absolute slope values

are presented as symbols in the left panel of Figure 2, where the right vertical axis is calibrated in terms of the transmittance change corresponding to the relative incident pulse energy of 0.8 (vertical arrow on the right panel of Figure 2).

From the fact that the "linearized" optical saturation spectrum is following closely the shape of the non-saturated (linear) absorption spectrum (shown by the solid lines) we may conclude that, under current conditions, the observed optical saturation effect is indeed linearly proportional to the molar extinction. Furthermore, such linear proportionality is observed for both samples i.e. independent of the C_M value. Figure 3 shows the ratio between the linear fit slope parameters

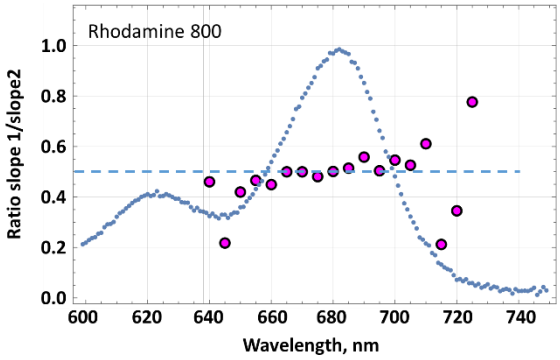

Figure 3. Ratio of the nonlinear transmittance spectra of Rhodamine 800 in ethanol at to different concentrations (magenta disks). The dashed line shows the known ratio of 0.5 for the concentrations. The fine-dotted curve indicates the relative absorption spectrum of the solution. Ratio values around peak absorption wavelengths coincide well with concentration ratio indicating that the nonlinear transmittance method is best applicable at sufficiently high-absorbing wavelengths.

(or NLT spectra) obtained at the two concentrations. This ratio is close to the factor 0.5, which coincides with the ratio between the corresponding concentrations. Therefore, if we consider that one of the samples represents a reference with known T_{lin} (or *O.D.*) spectrum and known value of ε_M (or C_M), then by measuring the T_{NL} spectrum in the reference sample and some other samples using identical nonlinear pulse excitation conditions, where the T_{lin} (or O.D.) for each sample is known, one may determine the ε_M or C_M values of the unknown chromophores. For example, if we take the higher-concentration solution to act as reference, and the lower-concentration solution as the one to be investigated, then from the fact that both samples show the same T_{lin} versus T_{NL} ratio we conclude that the corresponding ε_M values must be identical. In other words, if two solutions have identical optical path lengths and are measured under identical excitation conditions, but show different T_{lin} versus T_{NL} ratios, then this means that their ε_M values also scale in proportion to those ratios.

CONCLUSIONS

We have presented a proof-of-principle experiment where we show that by measuring optical saturation under identical femtosecond pulse excitation conditions in two different solutions, where the molar extinction coefficient of one of the solutions is known, this may be used to determine the ε_M value of the unknown solution. Two key requirements for such measurement are that, firstly, both the linear absorbance as well as the saturation-induced absorbance change are relatively small in both samples and, secondly, that the linearized nonlinear transmittance function is obtained from the experiment with sufficiently high degree of accuracy allowing the corresponding slope parameter to be reliably determined.

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